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Haematological Alterations Associated with Naturally Occurring Nephropathogenic Infectious Bronchitis in Broiler Chickens

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A B S T R A C T

Nephropathogenic Infectious Bronchitis (NIB) is an important viral disease of poultry associated with severe renal damage and economic losses. The present study investigated the haematological alterations associated with naturally occurring NIB in broiler chickens from five confirmed flocks. Gross and histopathological examination revealed visceral uricosis, renal congestion, haemorrhage, necrosis, interstitial nephritis, glomerulonephritis, tubular degeneration and necrosis, and urate crystal deposition. Haematological analysis showed significantly reduced ($P<0.05$) haemoglobin (Hb), total erythrocyte count (TEC), and total leukocyte count (TLC), indicating anaemia and leukopenia. Differential leukocyte count revealed significant heterophilia, lymphopenia, and reduced monocyte percentage, whereas eosinophil and basophil percentages did not differ significantly. These findings indicate that NIB is associated with characteristic haematological alterations that may serve as supportive indicators of disease in naturally infected broiler flocks.

Introduction

The emergence of NIB virus variants has raised serious concerns in the poultry industry due to their increased virulence and ability to cause severe renal damage, resulting in high morbidity, mortality, and economic losses (Bayry *et al.,* 2005; Li *et al.,* 2022). Unlike the classical respiratory form, nephropathogenic strains exhibit a marked tropism for the kidneys, leading to nephritis, urate deposition, and impaired renal function

(Balasubramaniam *et al.,* 2012; Li *et al.,* 2022). Several researchers have documented characteristic gross and histopathological renal lesions associated with NIB, including visceral uricosis, nephritis, tubular degeneration and necrosis, and urate crystal deposition (El-Hamid *et al.,* 2016; Timurkaan *et al.,* 2023; Patel *et al.,* 2026). However, information on the haematological alterations associated with naturally occurring NIB under field conditions remains limited (Yohannes *et al.,* 2013; Yadav *et al.,* 2021). Therefore, the present study was undertaken to investigate the haematological changes associated with naturally occurring NIB in broiler chickens, in cases confirmed by characteristic gross and histopathological lesions.

Materials and Methods

Sample Collection

The study was carried out at the Department of Veterinary Pathology, College of Veterinary Science, AVFU, Khanapara, Guwahati-22. Carcasses of birds suspected of NIB were received from disease outbreak areas and organized commercial broiler farms of different districts of Assam for post-mortem examination. Representative tissue samples from affected kidneys were collected in 10% neutral buffered formalin for histopathological examination and were routinely processed and stained with Haematoxylin and Eosin (H&E) stain. Based on the gross and histopathological findings, five flocks were confirmed to be affected with NIB and were included in the study. Approximately 2 ml of blood was collected from the wing vein of clinically affected birds from the confirmed flocks in EDTA vials for routine haematological examination. Blood samples were also collected from apparently healthy birds and maintained in EDTA vials for comparison.

Haematological estimation

Total Erythrocyte Count (TEC) and Total Leukocyte Count (TLC)

A modified staining technique based on Tsai *et al.,* (2014), which was originally developed for red-eared slider turtles was used for TEC and TLC. The staining solution was formulated by mixing 0.1 ml of HiMedia® Gram's crystal violet (catalogue no. S012) solution with 9.9 ml of 0.45% sodium chloride solution. 10 µl of chicken blood and 740 µl of staining solution were carefully mixed for cell counting, and the mixture was incubated for five minutes. After charging the hemacytometer with the stained sample, it was then allowed to stand for 10 minutes.

Red blood cells (RBCs) were counted in four small corner squares plus the centre square (1/25 mm^2), while white blood cells (WBCs) were counted in four big corner squares (1 mm^2). Cell counts were calculated using the following formulas:

1. **Total WBC count** = Total haematocytometer count ÷ 4 (large squares) ÷ 0.1 (depth of the chamber) x 75 (dilution rate).
2. **Total RBC count** = Total haemocytometer count x 5 (to reach 1-mm^2 area) ÷ 0.1 (depth of the chamber) x 75 (dilution rate).

Haemoglobin (Hb)

Sahli's apparatus was used to measure the Hb concentration using the acid hematin method, as detailed by Schalm *et al.,* (1975).

Differential leukocyte count (DLC)

DLC was performed in accordance with Schalm *et al.,* (1975).

Statistical Analysis

Student's t-test (Gosset, 1908) was used in SPSS software version 20 to statistically analyse the results from the haematological examination. P-values less than 0.05 (two-tailed) were deemed significant. The values for each parameter were presented as Mean ± SE.

Results and Discussion

Gross and Histopathology

Gross examination of NIB-affected birds revealed visceral uricosis with urate deposition on visceral organs (Figure 1) and severe renal involvement characterized by congestion, haemorrhage, necrosis (Figure 2), urate accumulation, and ureteral distension. Histopathologically (Figure 3), the kidneys showed interstitial nephritis, glomerulonephritis, tubular degeneration and necrosis, mononuclear cell infiltration, urate crystal deposition, and tubular epithelial

hyperplasia. Similar gross and microscopic lesions have been reported by Munuswamy *et al.,* (2021), Timurkaan *et al.,* (2023) and Patel *et al.,* (2026).

Haematological studies

Four haematological parameters were examined in the blood samples: Haemoglobin (Hb), Total Erythrocyte Count (TEC), Total Leukocyte Count (TLC), and Differential Leukocyte Count (DLC). The mean values of these parameters along with their standard error (SE), for apparently healthy group and NIB-affected group has been presented in Table 1.

Haemoglobin, Total Erythrocyte Count and Total Leukocyte Count

The mean Hb and TEC values were significantly reduced ($P<0.05$) in the NIB-affected group (8.98 ± 0.28 g/dl and 1.81 ± 0.04 × 10^6/µl, respectively) than in the apparently healthy group (11.00 ± 0.23 g/dl and 2.29 ± 0.05 × 10^6/µl, respectively), suggesting anaemia, which is in agreement with the findings of Yadav *et al.,* (2021). Leukopenia was indicated by significantly reduced ($P<0.05$) mean TLC values (12.00 ± 0.35 × 10^3/µl) in the NIB-affected group compared to the apparently healthy group (16.96 ± 0.49 × 10^3/µl). Yohannes *et al.,* (2013) and Yadav *et al.,* (2021) reported similar findings.

Differential Leukocyte count

Heterophilia was indicated by a significantly higher ($P<0.05$) mean percentage of heterophils in the NIB-affected group (17.40 ± 0.58%) than in the apparently healthy group (10.90 ± 0.48%), which is in agreement with the findings of Yohannes *et al.,* (2013) and Yadav *et al.,* (2021). The mean percentage of lymphocytes was significantly reduced ($P<0.05$) in the NIB-affected group (73.40 ± 0.42%) compared to the apparently healthy group (78.00 ± 0.66%), indicating lymphopenia, which corroborates the findings of Yohannes *et al.,* (2013) and Yadav *et al.,* (2021). The mean percentage of monocytes was significantly reduced ($P<0.05$) in the NIB-affected group (8.50 ± 0.45%) compared to the apparently healthy group (10.20 ± 0.55%); however, Yohannes *et al.,* (2013) reported no significant difference, whereas Yadav *et al.,* (2021) observed a significant increase in monocyte counts. There was no significant difference ($P>0.05$) in the mean percentages of eosinophils and basophils between the NIB-affected group (0.40 ± 0.69% and 0.30 ± 0.15%, respectively) and the apparently healthy group (0.50 ± 0.16% and 0.40 ± 0.16%, respectively), which is consistent with the findings of Yohannes *et al.,* (2013) and Yadav *et al.,* (2021).

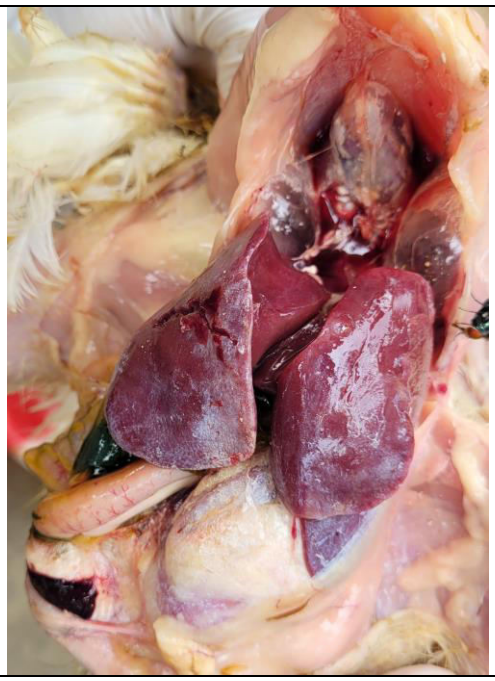

Figure.1 Carcasses showing deposition of urates on visceral surfaces

Figure.2 Kidneys showing areas of necrosis

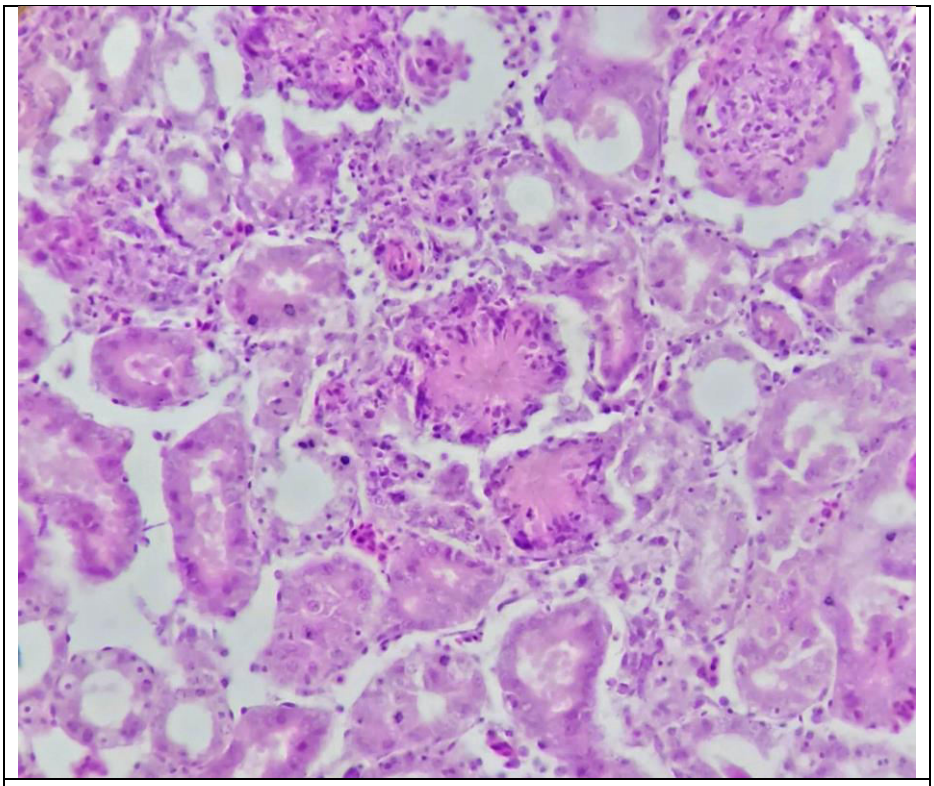

Figure.3 Photomicrograph of kidney revealed urate crystal deposition in renal tubules, tubular necrosis and interstitial nephritis (H&E, 40X)

Table.1 Mean ± SE of different haematological parameters of NIB-affected birds and apparently healthy birds' group

S.N.	Parameters	NIB-affected	Apparently healthy
1.	Hb (g/dl)	8.98* ± 0.28	11.00 ± 0.23
2.	TEC (10^6/µl)	1.81* ± 0.04	2.29 ± 0.05
3.	TLC (10^3/µl)	12.00* ± 0.35	16.96 ± 0.49
4.	DLC (%)		
a.	Heterophils	17.40* ± 0.58	10.90 ± 0.48
b.	Lymphocytes	73.40* ± 0.42	78.00 ± 0.66
c.	Monocytes	8.50* ± 0.45	10.20 ± 0.55
d.	Eosinophils	0.40 ± 0.69	0.50 ± 0.16
e.	Basophils	0.30 ± 0.15	0.40 ± 0.16

P<0.05 = *; P>0.05 = non-significant. Means bearing superscript indicate a statistically significant difference (P<0.05).

In conclusion, NIB in broiler chickens was characterized by visceral uricosis and severe renal lesions including interstitial nephritis, glomerulonephritis, tubular degeneration and necrosis, mononuclear cell infiltration, and urate crystal deposition.

Haematological examination revealed anaemia, leukopenia, heterophilia, lymphopenia, and a significant reduction in monocyte percentage in affected birds.

These findings suggest that haematological evaluation may provide useful supportive information in the diagnosis and assessment of NIB under field conditions. In addition, the modified staining technique described by Tsai *et al.,* (2014), originally developed for red-eared slider turtles, was successfully adapted in the present

study for estimation of TEC and TLC in chickens, highlighting its potential utility for avian haematological investigations.

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Author Contributions

Dr. Sakshi Patel: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Writing – original draft preparation, Writing – review & editing, Visualization. Dr. Biswajit Dutta: Supervision, Conceptualization, Validation, Methodology, Writing – review & editing. Dr. Pankaj Deka: Resources, Methodology, Investigation, Provision of experimental material (stain), Validation. Dr. S. A. Begum: Investigation, Resources, Validation, Supervision support. Dr. Rupam Dutta: Advisory support, Validation, Review of experimental design and manuscript. Dr. Abhijit Deka: Methodology support, Validation, Supervision. Dr. Suchand Doloi: Investigation, Data collection (sample collection support), Validation. Dr. Ajay Mendake: Writing – review & editing, Visualization support, Manuscript assistance.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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